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(54) **COMPOSITIONS AND METHODS FOR RECOVERY OF NUCLEIC ACIDS OR PROTEINS FROM TISSUE SAMPLES FIXED IN CYTOLOGY MEDIA**

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See application file for complete search history.

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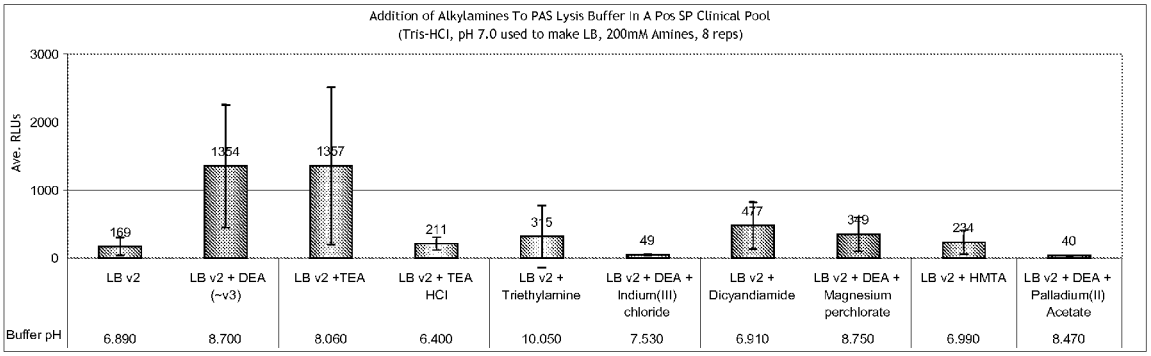
(57) **ABSTRACT**

The present invention provides compositions and methods for improving nucleic acid or protein recovery from fixed biological samples.

33 Claims, 9 Drawing Sheets



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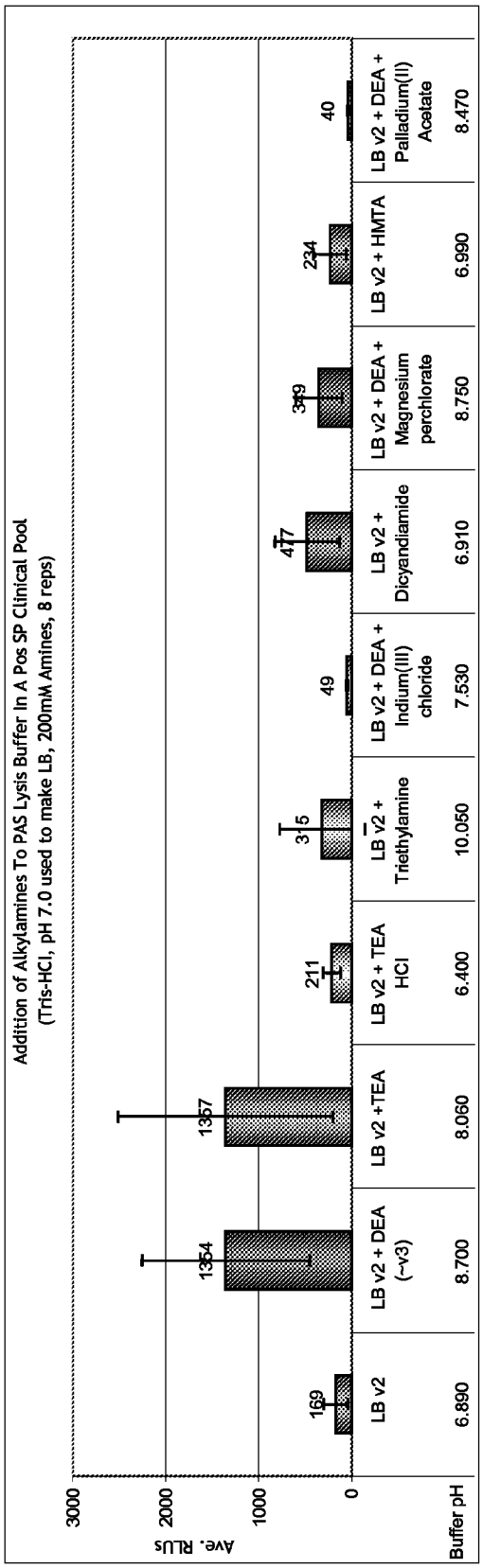
Addition of Alkylamines To PAS Lysis Buffer In A Pos SP Clinical Pool
(Tris-HCl, pH 7.0 used to make LB, 200mM Amines, 8 reps)
Ave. RLUs
3000
2000
1000
0
169
1354
1357
211
315
49
417
319
224
40
Buffer pH
LB v2
6.890
LB v2 + DEA (~v3)
8.700
LB v2 +TEA
8.060
LB v2 + TEA HCl
6.400
LB v2 + Triethylamine
10.050
LB v2 + DEA + Indium(III) chloride
7.530
LB v2 + Dicyandiamide
6.910
LB v2 + DEA + Magnesium perchlorate
8.750
LB v2 + HMTA
6.990
LB v2 + DEA + Palladium(II) Acetate
8.470

Fig. 1A

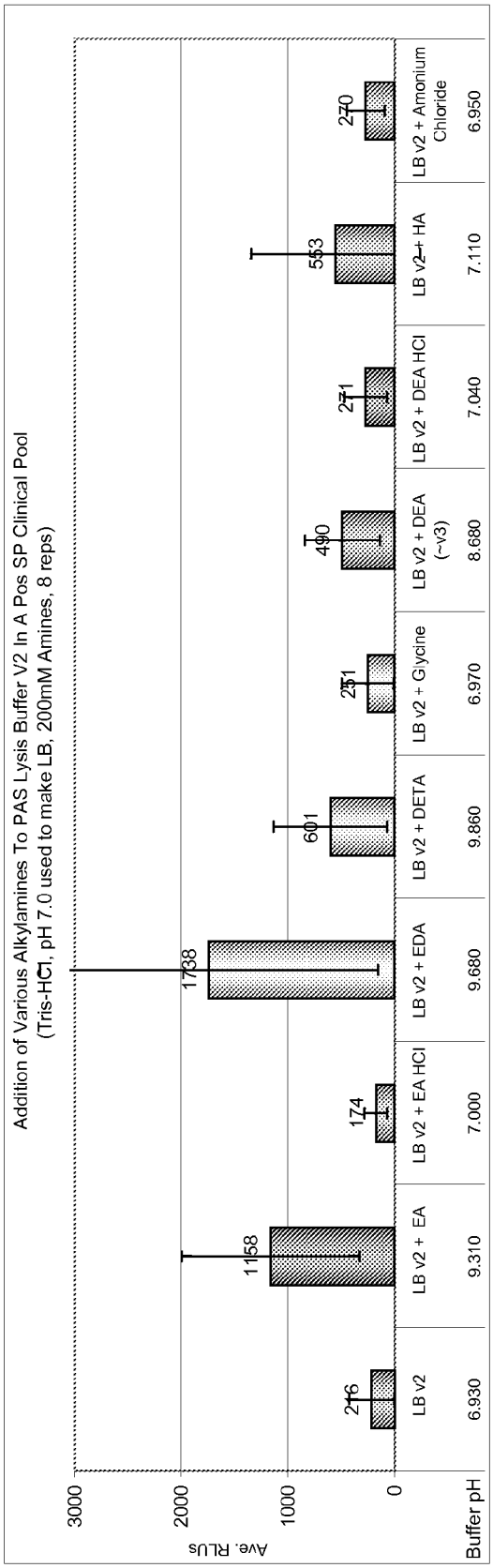
Addition of Various Alkylamines To PAS Lysis Buffer V2 In A Pos SP Clinical Pool
(Tris-HCl, pH 7.0 used to make LB, 200mM Amines, 8 reps)
Ave. RLUs
3000
2000
1000
0
216
1158
174
1738
601
251
490
271
553
270
LB v2
LB v2 + EA
LB v2 + EA HCl
LB v2 + EDA
LB v2 + DETA
LB v2 + Glycine
LB v2 + DEA (~v3)
LB v2 + DEA HCl
LB v2 + HA
LB v2 + Amonium Chloride
Buffer pH
6.930
9.310
7.000
9.680
9.860
6.970
8.680
7.040
7.110
6.950

Fig. 1B

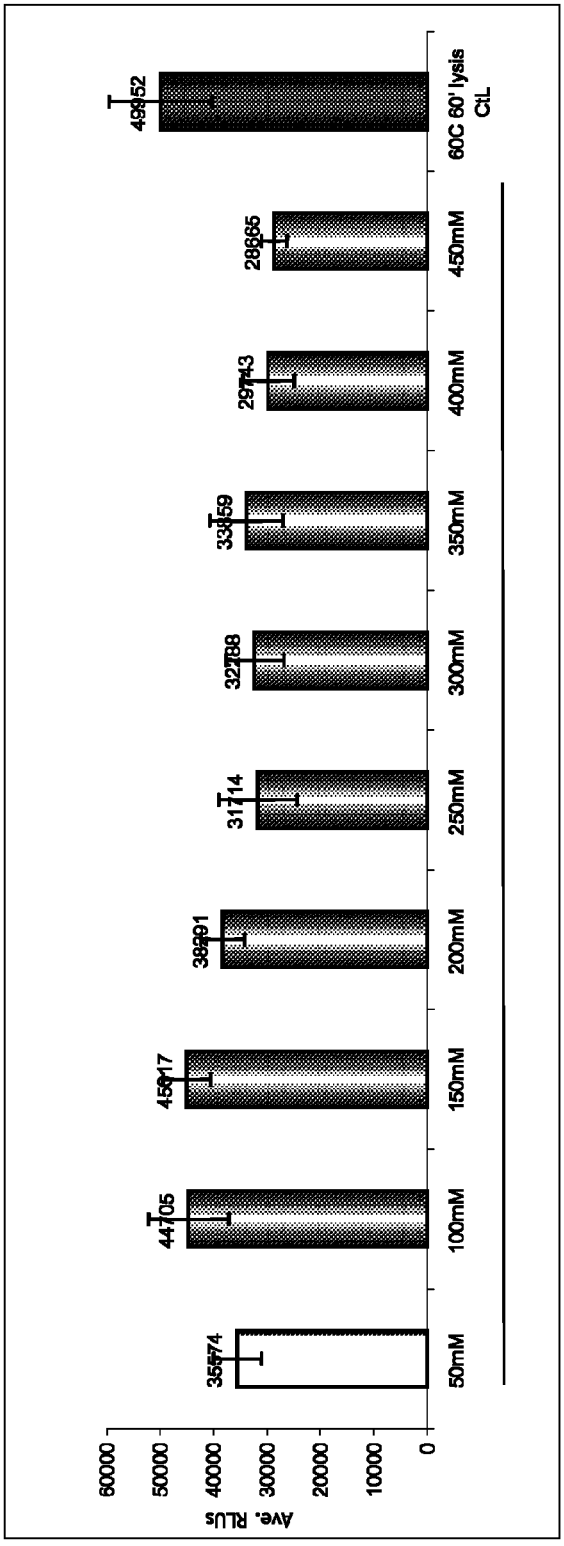
Ave. RLUs
60000
50000
40000
30000
20000
10000
0
50mM
100mM
150mM
200mM
250mM
300mM
350mM
400mM
450mM
60C 60' lysis CtL

Fig. 2

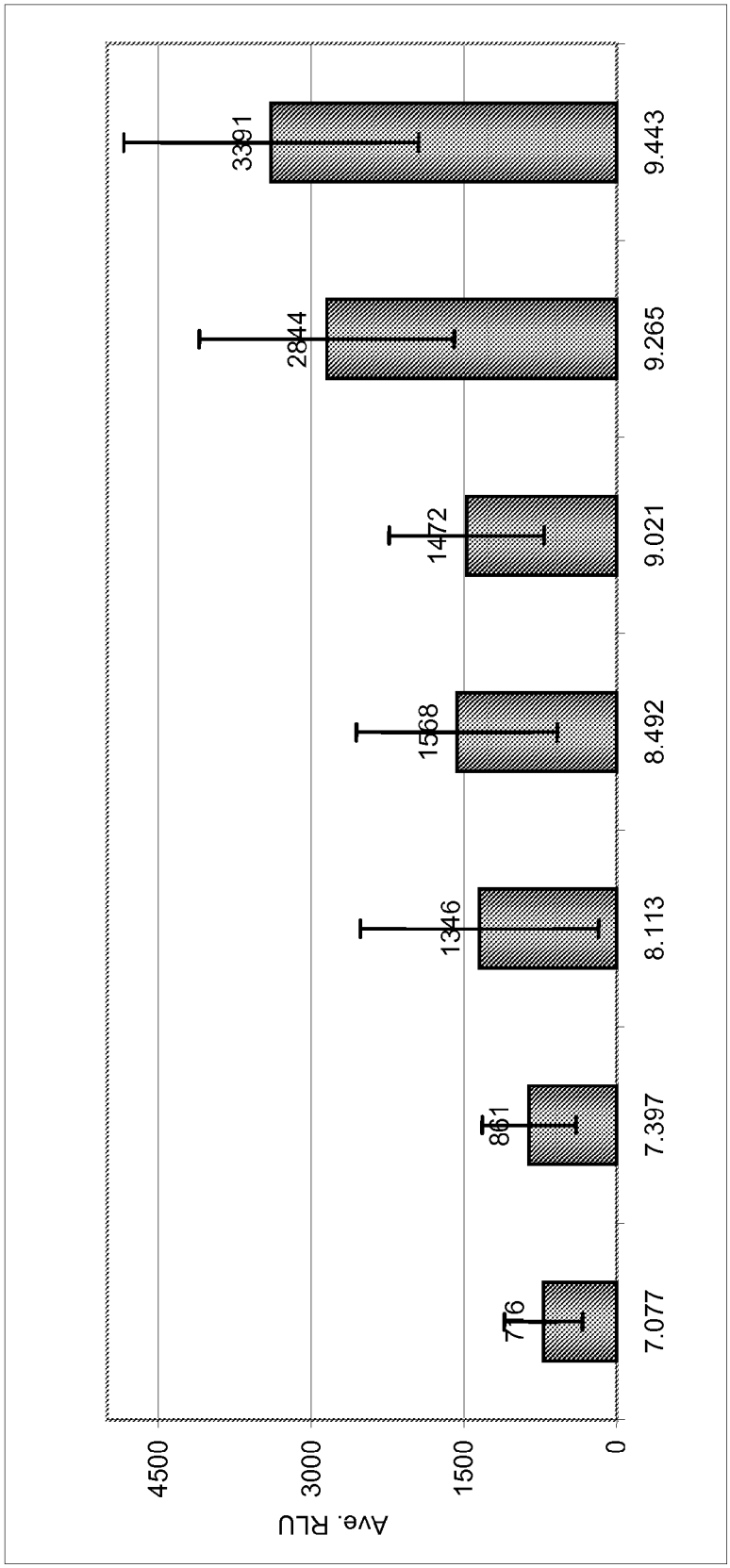
Ave. RLU
4500
3000
1500
0
3391
2844
1472
1568
1346
861
776
9.443
9.265
9.021
8.492
8.113
7.397
7.077

FIG. 3

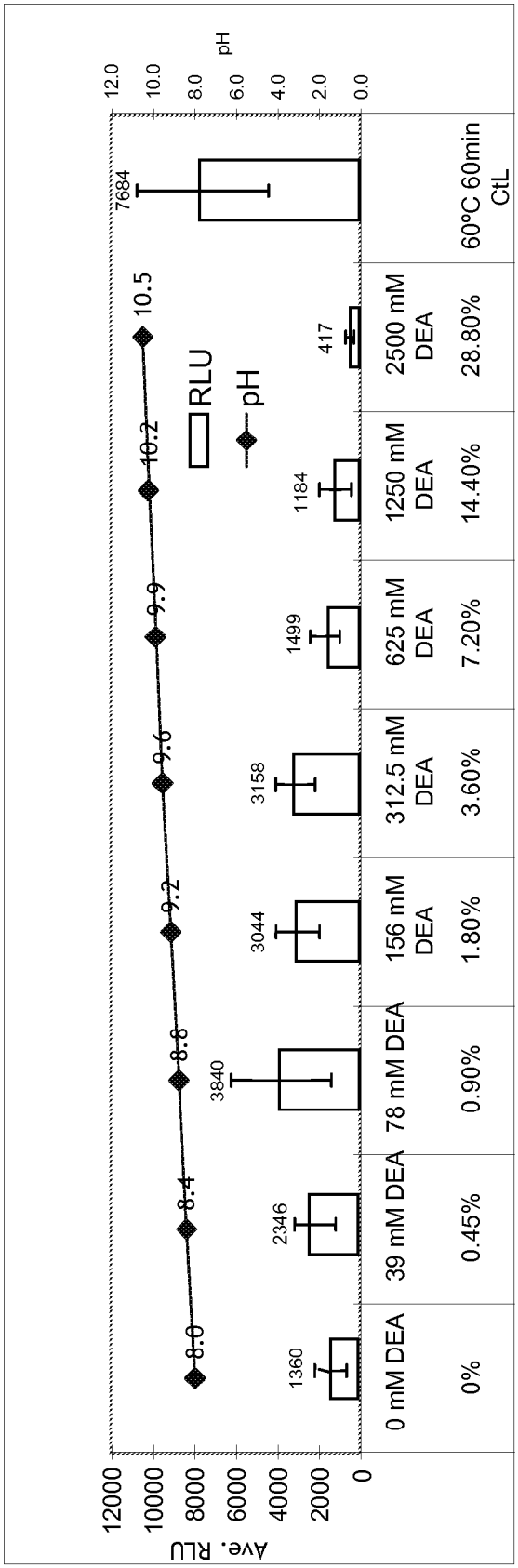
Ave. RLU
12000
10000
8000
6000
4000
2000
0
pH
12.0
10.0
8.0
6.0
4.0
2.0
0.0
RLU
pH
1360
2346
3840
3044
3158
1499
1184
417
7684
8.0
8.4
8.8
9.2
9.6
9.9
10.2
10.5
0 mM DEA
0%
39 mM DEA
0.45%
78 mM DEA
0.90%
156 mM DEA
1.80%
312.5 mM DEA
3.60%
625 mM DEA
7.20%
1250 mM DEA
14.40%
2500 mM DEA
28.80%
60°C 60min CtL

FIG. 4

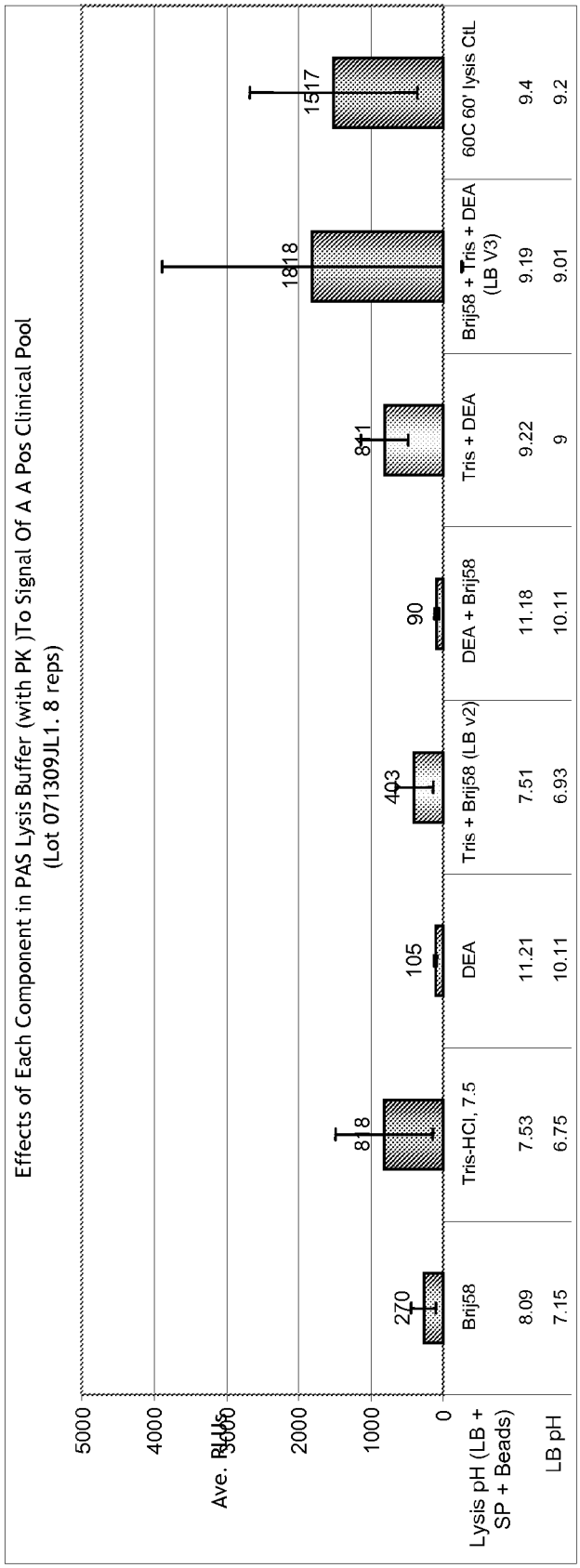
Effects of Each Component in PAS Lysis Buffer (with PK)To Signal Of A A Pos Clinical Pool
(Lot 071309JL1. 8 reps)
Ave. RLUs
5000
4000
3000
2000
1000
0
270
818
105
403
90
841
1818
1517
Lysis pH (LB + SP + Beads)
LB pH
Brij58
8.09
7.15
Tris-HCl, 7.5
7.53
6.75
DEA
11.21
10.11
Tris + Brij58 (LB v2)
7.51
6.93
DEA + Brij58
11.18
10.11
Tris + DEA
9.22
9
Brij58 + Tris + DEA (LB V3)
9.19
9.01
60C 60' lysis CtL
9.4
9.2

FIG. 5

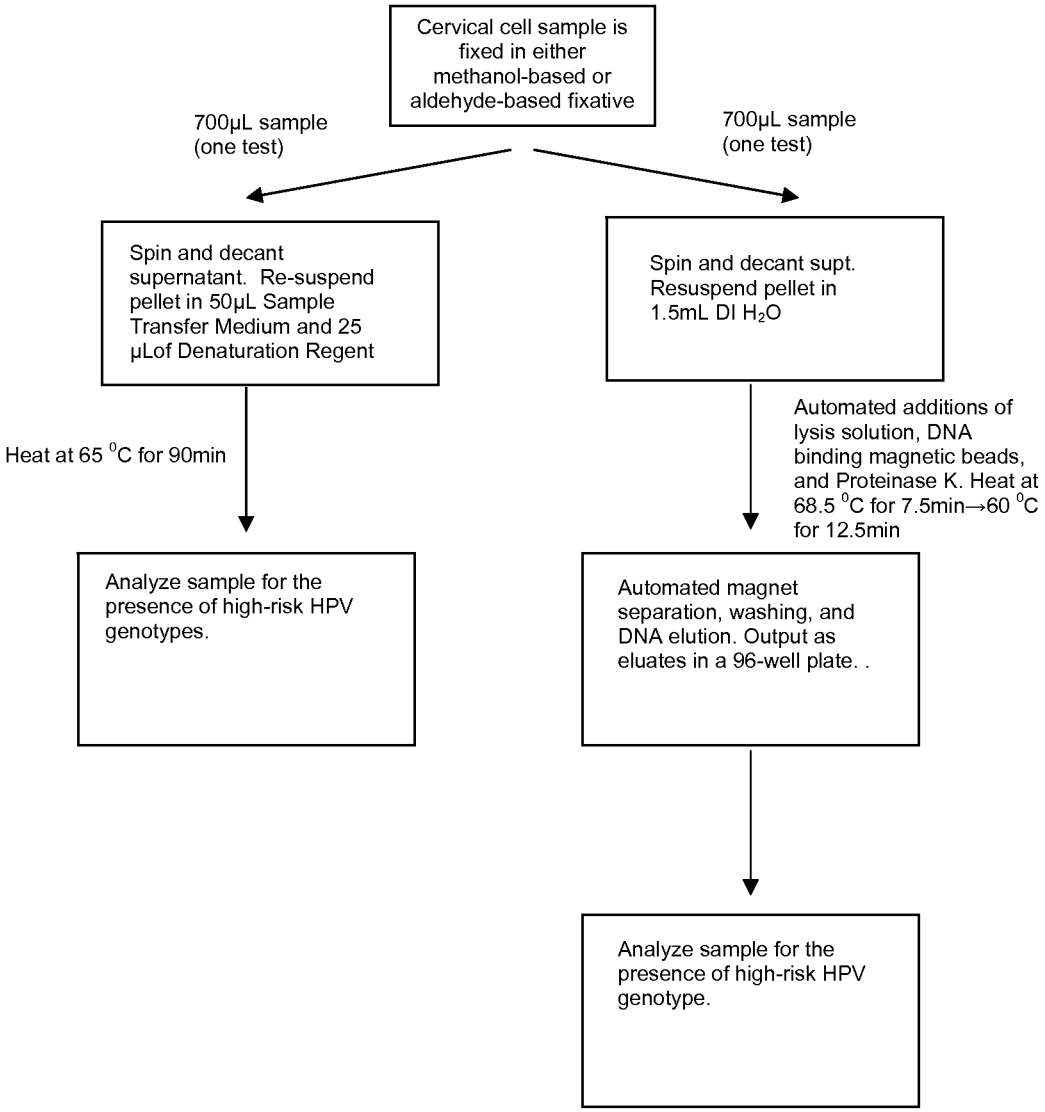
Cervical cell sample is fixed in either methanol-based or aldehyde-based fixative
700µL sample (one test)
700µL sample (one test)
Spin and decant supernatant. Re-suspend pellet in 50µL Sample Transfer Medium and 25 µLof Denaturation Regent
Spin and decant supt. Resuspend pellet in 1.5mL DI H_2O
Heat at 65 ^{0}C for 90min
Automated additions of lysis solution, DNA binding magnetic beads, and Proteinase K. Heat at 68.5 ^{0}C for 7.5min→60 ^{0}C for 12.5min
Analyze sample for the presence of high-risk HPV genotypes.
Automated magnet separation, washing, and DNA elution. Output as eluates in a 96-well plate. .
Analyze sample for the presence of high-risk HPV genotype.

FIG. 6

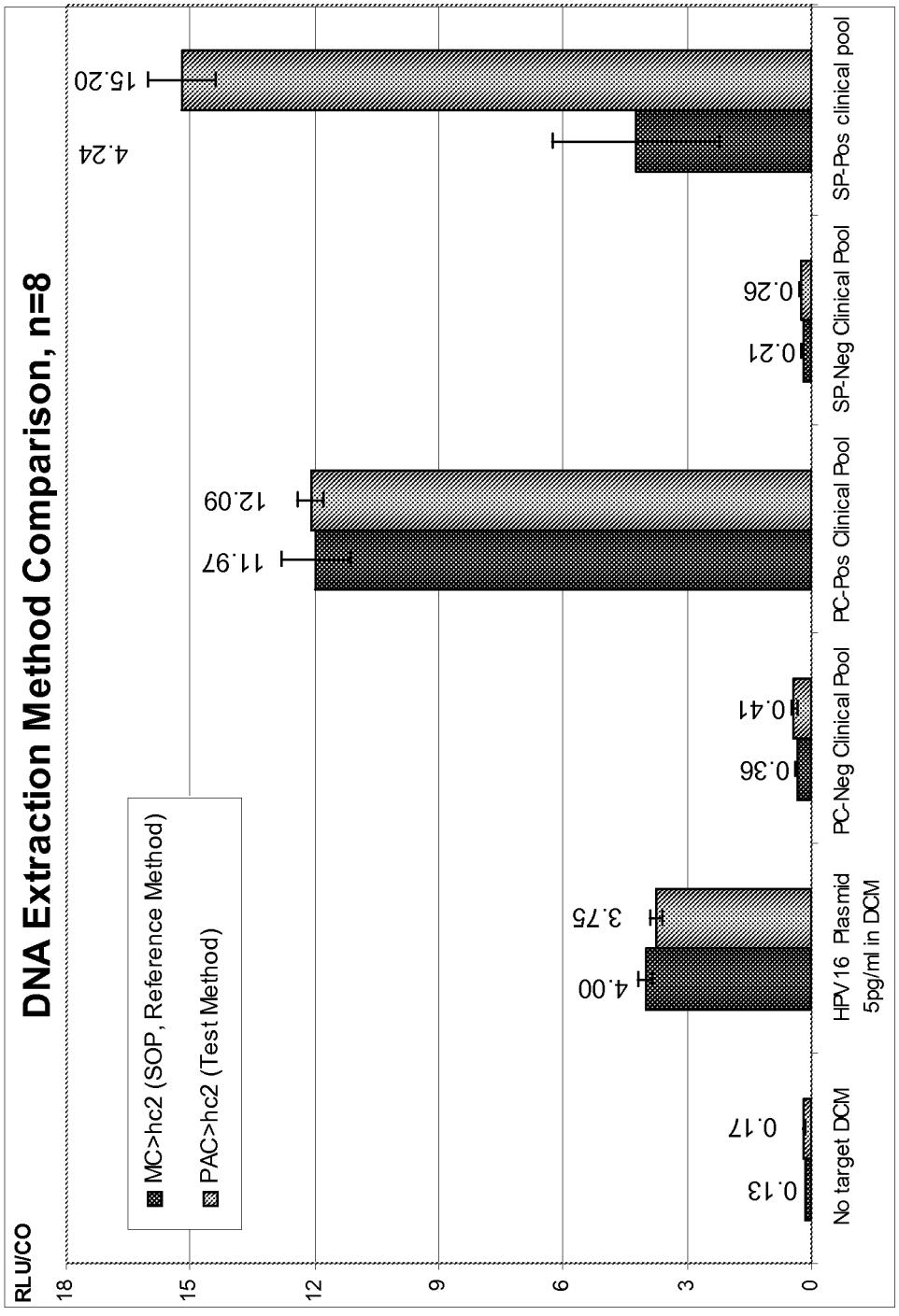
DNA Extraction Method Comparison, n=8
RLU/CO
MC>hc2 (SOP, Reference Method)
PAC>hc2 (Test Method)
0
3
6
9
12
15
18
0.13
0.17
3.75
4.00
0.36
0.41
11.97
12.09
0.21
0.26
4.24
15.20
No target DCM
HPV16 Plasmid 5pg/ml in DCM
PC-Neg Clinical Pool
PC-Pos Clinical Pool
SP-Neg Clinical Pool
SP-Pos clinical pool

FIG. 7

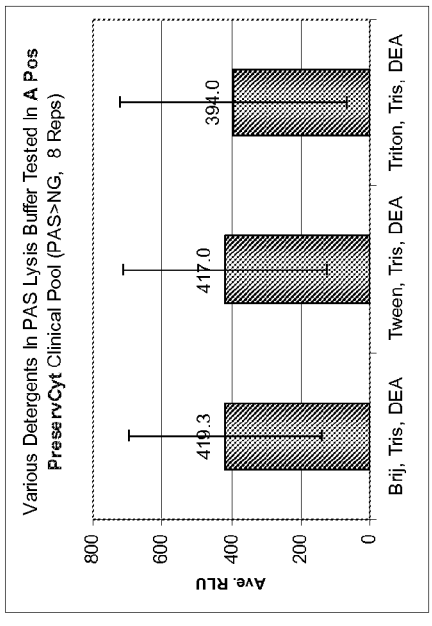

FIG. 8A

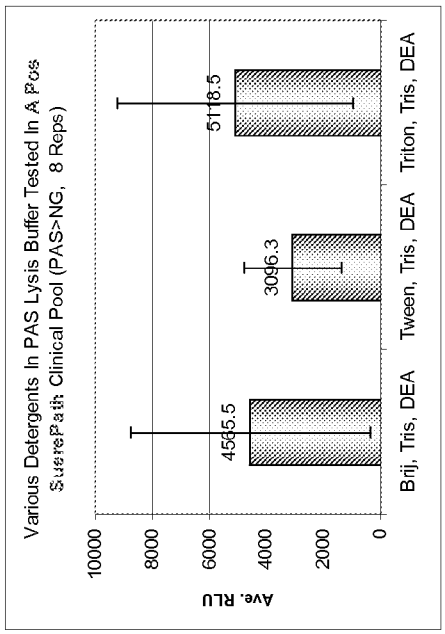

FIG. 8B

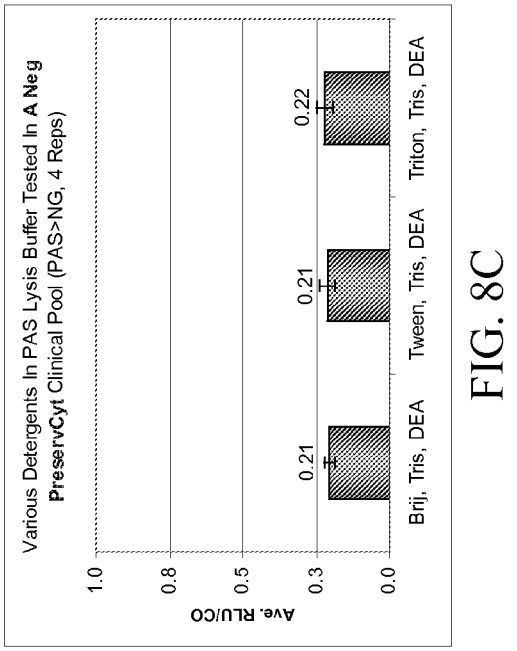

FIG. 8C

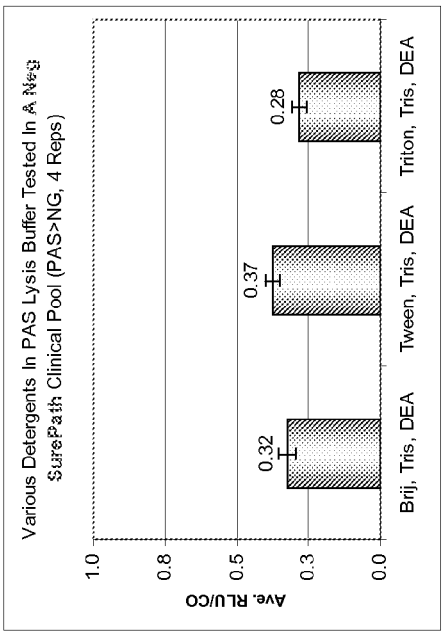

FIG. 8D

COMPOSITIONS AND METHODS FOR RECOVERY OF NUCLEIC ACIDS OR PROTEINS FROM TISSUE SAMPLES FIXED IN CYTOLOGY MEDIA

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority from U.S. Provisional Patent Application Ser. No. 61/242,258 (filed Sep. 14, 2009) and U.S. Provisional Patent Application Ser. No. 61/253,300 (filed Oct. 20, 2009), the contents of which are incorporated herein by reference in their entireties. A PCT application entitled "Compositions And Methods For Recovery Of Nucleic Acids Or Proteins From Tissue Samples Fixed In Cytology Media" (filed concurrently herewith on Sep. 14, 2010) is also incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

Compositions, methods, and kits for improved recovery of nucleic acids or proteins from fixed biological samples are described.

BACKGROUND OF THE INVENTION

In the fields of histology, pathology, and cell biology, fixation is a chemical process by which biological samples are preserved from decay. Fixation terminates any ongoing biochemical reactions, and may also increase the mechanical strength or stability of the treated samples. The purpose of fixation is to preserve a sample of biological material as close to its natural state as possible. Fixed samples are used for examination or analysis.

Fixatives can be classified as cross-linking or precipitating fixatives.

Cross-linking fixatives act by creating covalent chemical bonds between proteins in tissue. This anchors soluble proteins to the cytoskeleton, and lends additional rigidity to the tissue. Aldehydes are by far the most commonly used cross-linking fixatives. Although aldehyde-fixed biological samples are useful for histological, pathological, and cell biological applications, they pose several problems for molecular analysis of the preserved sample. For example, fixation with aldehydes causes protein-protein, DNA-protein, and RNA-protein cross-links to form, which interferes with the ability to extract and purify proteins and nucleic acids. Moreover, reversal of cross-linking often results in free aldehyde in the sample, which can interfere with functional proteins (such as enzymes or antibodies), nucleic acid probes, resins, or any other functional reagents with amino groups that are used in sample processing and analysis.

As such, there remains a need for methods and compositions that increase the efficiency of isolating various components (such as nucleic acids, proteins, and organelles) from biological samples fixed in fixed in aldehyde-based cytology media.

Precipitating fixatives act by reducing the solubility of protein molecules and disrupting hydrophobic interactions. As this process is very different from cross-linking fixation, biological samples fixed with precipitating fixatives often must be processed with different reagents and methods than those used with cross-linking fixatives. Alcohols are commonly used precipitating fixatives. There is a need for methods and compositions that increase the efficiency of isolating various components (such as nucleic acids, proteins, and organelles) from biological samples fixed in alcohol-based cytology media.

Therefore, there remains is a need for methods and reagents that are useful in extracting various components from fixed biological samples (such as nucleic acids, proteins, and organelles), regardless of the type fixative used. In particular, lysis solutions are needed that may be used for biological samples fixed in cytology media that is cross-linking-based, precipitating-based, or both.

SUMMARY OF THE INVENTION

The present disclosure provides a lysis composition that can be used to lyse biological samples fixed in cytology media. The cytology medium can comprise either precipitating or cross-linking fixatives, or both.

The present disclosure also provides methods of preparing a fixed biological sample for analysis comprising lysing the fixed biological sample in the presence of a buffered composition. The lysing process creates a lysate, from which a component can be isolated. The isolated component can be subjected to analysis.

The methods and compositions disclosed herein exhibit improved extraction of biological samples regardless of the fixative used in the cytology medium.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. **1**A and **1**B show the effects of various amine-containing compounds on the extraction of DNA from aldehyde-fixed clinical cervical samples using a lysis solution comprising 150 mM Tris. Columns represent average relative light unit (RLU), with error bars representing the standard deviation of the eight replicates tested. The pH of each lysis solution is also displayed. Abbreviations: LB v2=lysis solution of: (1) 3% (v/v) Brij-58, and (2) 150 mM Tris-HCl; DEA=diethanolamine; TEA=triethanolamine; TEA HCl=triethanolamine hydrogen chloride; HMTA=hexamethylene-tetramine; EA=ethanolamine; EDA=ethylenediamine; DETA=diethylenetriamine; DEA HCl=diethanolamine hydrogen chloride. Typically, 1.5 mL of sample is added to 1 mL of lysis buffer, plus 25 μl of Proteinase K (10 mg/ml stock) and 60 μl of 1.5% (v/v) AxpH™ DNA-affinity magnetic beads.

FIG. **2** shows the effect of varying the concentration of Tris-HCl on the efficiency of a lysis solution comprising 300 mM diethanolamine. The labels in the x-axis of the graph indicate the concentration of Tris-HCl in each sample. Columns represent average relative light unit (RLU), with error bars representing the standard deviation of the eight replicates tested.

FIG. **3** shows the effect of pH on HPV DNA detection in DNA isolated from aldehyde-fixed cervical samples using a lysis solution comprising 150 mM Tris and 300 mM diethanolamine. The labels in the x-axis of the graph indicate the pH of the lysis solution used. Columns represent average relative light unit (RLU), with error bars representing the standard deviation of the eight replicates tested.

FIG. **4** shows the effect of varying the concentration of diethanolamine on HPV DNA detection in DNA isolated from aldehyde-fixed cervical samples using a lysis solution comprising 150 mM Tris. The top table shows raw data from each replicate for each lysis solution tested. The lower table shows combined data for each lysis solution tested and is displayed graphically in the bar graph at the bottom. The labels in the x-axis of the graph indicate the pH of the lysis

solution used. Columns represent average relative light unit (RLU), with error bars representing the standard deviation of the eight replicates tested. The pH of each lysis solution is also displayed on the graph. Control conditions are the same as the test conditions absent the diethanolamine.

FIG. **5** shows the effects of detergent, Tris, and diethanolamine on extraction and detection of HPV DNA in aldehyde-fixed cervical samples. The top table shows raw data from each replicate for each lysis solution tested. The lower table shows combined data for each lysis solution tested and is displayed graphically in the bar graph at the bottom. The labels in the x-axis of the graph indicate the pH of the lysis solution used. Columns represent average relative light unit (RLU), with error bars representing the standard deviation of the eight replicates tested. The pH of each lysis solution is also displayed on the graph.

FIG. **6** is a flow chart showing the protocols used for determining the usefulness of lysis solutions in both manual and automated extraction of nucleic acid from either alcohol-based or aldehyde-based lysis buffers. The left branch of the flow chart shows a manual method, while the right branch shows an automated method using magnetic beads.

FIG. **7** shows the effectiveness of extracting nucleic acids using two types of lysis solutions. "MC" indicates manual conversion of the samples, as is currently performed with liquid based cytology samples and as indicated in FIG. **6**. "PAC" indicates pre-analytic chemistry. "PC-Neg Clinical Pool" indicates HPV-negative cervical clinical samples that have been fixed with PRESERVCYT liquid cytological preservation media. "PC-Pos Clinical Pool" indicates HPV-negative cervical clinical samples that have been spiked with HPV-positive SiHa cells and fixed with PRESERVCYT liquid cytological preservation media. "SP-Neg Clinical Pool" indicates HPV-negative cervical clinical samples that have been fixed with SUREPATH liquid cytological preservation media. "SP-Pos Clinical Pool" indicates HPV-negative cervical clinical samples that have been spiked with HPV-positive SiHa cells and fixed with SUREPATH liquid cytological preservation media. A negative control containing only Digene Collection Medium and a positive control containing HPV16 plasmid suspending in Digene Collection Medium also were tested.

FIGS. **8**A to **8**D show the effect of varying the detergent on extraction and detection of HPV DNA in samples preserved in PRESERVCYT or SUREPATH.

DETAILED DESCRIPTION OF THE INVENTION

The present disclosure relates to reagents and methods that are useful in universal protocols for extracting various components from biological samples fixed in a variety of fixative materials and amenable to high through-put automation.

In particular, the present disclosure provides a composition comprising a fixed biological sample and a lysis solution, the lysis solution comprising at least two amines.

As used herein, the term "fixed biological sample" refers to any biological material that has been preserved with a fixative agent, including but not limited to paraffin-embedded tissues or organs, tissue samples stored in liquid cryological preservation media, and cervical or gynecological swabs stored in liquid cryological preservation material. The fixative agent may be a cross-linking fixative agent or a precipitating fixative agent. Cross-linking fixatives include without limitation aldehydes (such as formaldehyde, paraformaldehyde, and glutaraldehyde), osmium tetroxide, potassium dichromate, chromic acid, and potassium permanganate. Precipitating fixative solutions include without limitation alcohols (such as ethanol and methanol) and acetic acid.

An example of an alcohol-based cytology medium is PRESERVCYT™. An example of an aldehyde-based cytology medium is SUREPATH™.

As used herein, the terms "lysis" and "lysing" refer to the act of disrupting the integrity of a cell wall; a cell membrane; or an organelle defined by a lipid membrane, including but not limited to endoplasmic reticulum, Golgi apparatus, lysosome, mitochondrion, nucleus, vacuole, and vesicle. Exemplary methods of lysis include mechanical lysis, such as by sonication or cytolysis; and chemical lysis, including use of detergents such as polyoxyethylene (20) cetyl ether (sold commercially as Brij-58), 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (sold commercially as CHAPS), NONIDET™ P-40 (also known as Igepal CA-630, tert-octylphenoxy poly(oxyethylene)ethanol), deoxycholate, TritonTRITON™ X-100 (also known as 4-(1,1,3,3-Tetramethylbutyl)phenyl-polyethylene glycol), sodium dodecyl sulfate (sold commercially as SDS), and/or polysorbate surfactants (sold commercially as TWEEN).

As used herein, "lysis solution" refers to any solution that is useful for lysing a cell. Exemplary lysis solutions include without limitation hypotonic lysis solutions and detergent-based lysis solutions, including but not limited to lysis solutions comprising polyoxyethylene (20) cetyl ether (sold commercially as Brij-58), 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (sold commercially as CHAPS), NONIDET P-40 (also known as Igepal CA-630, tert-octylphenoxy poly(oxyethylene)ethanol), deoxycholate, TRITON X-100 (also known as 4-(1,1,3,3-Tetramethylbutyl) phenyl-polyethylene glycol), sodium dodecyl sulfate (sold commercially as SDS), and/or polysorbate surfactants (sold commercially as TWEEN). The precise type and formulation of the lysis solution can be readily determined by a person having ordinary skill in the art according to the sample type, the method of lysis, the analyte of interest, and the method of analysis to be used.

Amines are derivatives of ammonia and are classified according to the number of hydrogens of ammonia replaced by organic groups. Primary amines are compounds having the formula of (RNH_2) wherein R is an organic group. Secondary amines are compounds having the formula of (R_2NH) wherein R is an organic group. Tertiary amines are compounds having the formula of (R_3N), wherein R is an organic group. Secondary and tertiary amines may also be cyclic molecules in which the nitrogen atom of the amine group is integral to the ring structure. Examples of amines include methylamine, dimethylamine, diethylamine, hydroxylamine (HA), trimethlylamine, triethylamine, monoethanolamine (EA), diethanolamine (DEA), triethanolamine (TEA), tris (hydroxymethyl)aminomethane (TRIS), ethylenediamine, diethylenetriamine (DETA) or hexamethylenetetramine (HMTA), aniline, and amino acids. Other examples of amines will be readily apparent to a person having ordinary skill in the art. Any amine may used in the compositions and methods disclosed herein.

In one embodiment, the amount of each amine in the lysis solution is selected from the group consisting of: about 25 mM or greater, about 50 mM or greater, about 100 mM or greater, about 150 mM or greater, about 200 mM or greater, 250 mM or greater, about 260 mM or greater, about 270 mM or greater, about 280 mM or greater, about 290 mM or greater, about 300 mM or greater, about 310 mM or greater, about 320 mM or greater, about 330 mM or greater, about 340 mM or greater, about 350 mM or greater, about 400 mM or greater, about 450 mM or greater, about 500 mM or greater, from

about 25 mM to about 100 mM, from about 25 mM to about 150 mM, from about 25 mM to about 200 mM, from about 25 mM to about 250 mM, from about 25 mM to about 260 mM, from about 25 mM to about 270 mM, from about 25 mM to about 280 mM, from about 25 mM to about 290 mM, from about 25 mM to about 300 mM, from about 25 mM to about 310 mM, from about 25 mM to about 320 mM, from about 25 mM to about 330 mM, from about 25 mM to about 340 mM, from about 25 mM to about 350 mM, from about 25 mM to about 400 mM, from about 25 mM to about 450 mM, from about 25 mM to about 500 mM, from about 50 mM to about 100 mM, from about 50 mM to about 150 mM, from about 50 mM to about 200 mM, from about 50 mM to about 250 mM, from about 50 mM to about 260 mM, from about 50 mM to about 270 mM, from about 50 mM to about 280 mM, from about 50 mM to about 290 mM, from about 50 mM to about 300 mM, from about 50 mM to about 310 mM, from about 50 mM to about 320 mM, from about 50 mM to about 330 mM, from about 50 mM to 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from about 310 mM to about 340 mM, from about 310 mM to about 350 mM, from about 310 mM to about 400 mM, from about 310 mM to about 450 mM, from about 310 mM to about 500 mM, from about 320 mM to about 330 mM, from about 320 mM to about 340 mM, from about 320 mM to about 350 mM, from about 320 mM to about 400 mM, from about 320 mM to about 450 mM, from about 320 mM to about 500 mM,), from about 330 mM to about 340 mM, from about 330 mM to about 350 mM, from about 330 mM to about 400 mM, from about 330 mM to about 450 mM, from about 330 mM to about 500 mM, from about 340 mM to about 350 mM, from about 340 mM to about 400 mM, from about 340 mM to about 450 mM, from about 340 mM to about 500 mM, from about 350 mM to about 400 mM, from about 350 mM to about 450 mM, from about 350 mM to about 500 mM, from about 400 mM to about 450 mM, from about 400 mM to about 500 mM, from about 450 mM to about 500 mM, about 100 mM, or about 150 mM, or about 200 mM, or about 250 mM, or about 260 mM, or about 270 mM, or about 280 mM, or about 290 mM, or about 300 mM, or about 310 mM, or about 320 mM, or about 330 mM, or about 340 mM, or about 350 mM, or about 400 mM, or about 450 mM, or about 500 mM.

In one embodiment, the amount of each amine in the lysis solution is selected from the group consisting of: from about 0.1% (w/v) to about 0.2% (w/v), from about 0.1% (w/v) to about 0.3% (w/v), from about 0.1% (w/v) to about 0.4% (w/v), from about 0.1% (w/v) to about 0.5% (w/v), from about 0.1% (w/v) to about 0.6% (w/v), from about 0.1% (w/v) to about 0.7% (w/v), from about 0.1% (w/v) to about 0.8% (w/v), from about 0.1% (w/v) to about 0.9% (w/v), from about 0.1% (w/v) to about 1.0% (w/v), from about 0.1% (w/v) to about 1.5% (w/v), from about 0.1% (w/v) to about 2.0% (w/v), from about 0.1% (w/v) to about 2.5% (w/v), from about 0.1% (w/v) to about 3% (w/v), from about 0.1% (w/v) to about 4% (w/v), from about 0.1% (w/v) to about 5% (w/v), from about 0.1% (w/v) to about 7% (w/v), from about 0.1% (w/v) to about 9% (w/v), from about 0.1% (w/v) to about 11% (w/v), from about 0.1% (w/v) to about 13% (w/v), from about 0.1% (w/v) to about 15% (w/v), from about 0.2% (w/v) to about 0.3% (w/v), from about 0.2% (w/v) to about 0.4% (w/v), from about 0.2% (w/v) to about 0.5% (w/v), from about 0.2% (w/v) to about 0.6% (w/v), from about 0.2% (w/v) to about 0.7% (w/v), from about 0.2% (w/v) to about 0.8%

(w/v), from about 0.2% (w/v) to about 0.9% (w/v), from about 0.2% (w/v) to about 1.0% (w/v), from about 0.2% (w/v) to about 1.5% (w/v), from about 0.2% (w/v) to about 2.0% (w/v), from about 0.2% (w/v) to about 2.5% (w/v), from about 0.2% (w/v) to about 3% (w/v), from about 0.2% (w/v) to about 4% (w/v), from about 0.2% (w/v) to about 5% (w/v), from about 0.2% (w/v) to about 7% (w/v), from about 0.2% (w/v) to about 9% (w/v), from about 0.2% (w/v) to about 11% (w/v), from about 0.2% (w/v) to about 13% (w/v), from about 0.2% (w/v) to about 15% (w/v), from about 0.3% (w/v) to about 0.4% (w/v), from about 0.3% (w/v) to about 0.5% (w/v), from about 0.3% (w/v) to about 0.6% (w/v), from about 0.3% (w/v) to about 0.7% (w/v), from about 0.3% (w/v) to about 0.8% (w/v), from about 0.3% (w/v) to about 0.9% (w/v), from about 0.3% (w/v) to about 1.0% (w/v), from about 0.3% (w/v) to about 1.5% (w/v), from about 0.3% (w/v) to about 2.0% (w/v), from about 0.3% (w/v) to about 2.5% (w/v), from about 0.3% (w/v) to about 3% (w/v), from about 0.3% (w/v) to about 4% (w/v), from about 0.3% (w/v) to about 5% (w/v), from about 0.3% (w/v) to about 7% (w/v), from about 0.3% (w/v) to about 9% (w/v), from about 0.3% (w/v) to about 11% (w/v), from about 0.3% (w/v) to about 13% (w/v), from about 0.3% (w/v) to about 15% (w/v), from about 0.4% (w/v) to about 0.5% (w/v), from about 0.4% (w/v) to about 0.6% (w/v), from about 0.4% (w/v) to about 0.7% (w/v), from about 0.4% (w/v) to about 0.8% (w/v), from about 0.4% (w/v) to about 0.9% (w/v), from about 0.4% (w/v) to about 1.0% (w/v), from about 0.4% (w/v) to about 1.5% (w/v), from about 0.4% (w/v) to about 2.0% (w/v), from about 0.4% (w/v) to about 2.5% (w/v), from about 0.4% (w/v) to about 3% (w/v), from about 0.4% (w/v) to about 4% (w/v), from about 0.4% (w/v) to about 5% (w/v), from about 0.4% (w/v) to about 7% (w/v), from about 0.4% (w/v) to about 9% (w/v), from about 0.4% (w/v) to about 11% (w/v), from about 0.4% (w/v) to about 13% (w/v), from about 0.4% (w/v) to about 15% (w/v), from about 0.5% (w/v) to about 0.6% (w/v), from about 0.5% (w/v) to about 0.7% (w/v), from about 0.5% (w/v) to about 0.8% (w/v), from about 0.5% (w/v) to about 0.9% (w/v), from about 0.5% (w/v) to about 1.0% (w/v), from about 0.5% (w/v) to about 1.5% (w/v), from about 0.5% (w/v) to about 2.0% (w/v), from about 0.5% (w/v) to about 2.5% (w/v), from about 0.5% (w/v) to about 3% (w/v), from about 0.5% (w/v) to about 4% (w/v), from about 0.5% (w/v) to about 5% (w/v), from about 0.5% (w/v) to about 7% (w/v), from about 0.5% (w/v) to about 9% (w/v), from about 0.5% (w/v) to about 11% (w/v), from about 0.5% (w/v) to about 13% (w/v), from about 0.5% (w/v) to about 15% (w/v), from about 0.6% (w/v) to about 0.7% (w/v), from about 0.6% (w/v) to about 0.8% (w/v), from about 0.6% (w/v) to about 0.9% (w/v), from about 0.6% (w/v) to about 1.0% (w/v), from about 0.6% (w/v) to about 1.5% (w/v), from about 0.6% (w/v) to about 2.0% (w/v), from about 0.6% (w/v) to about 2.5% (w/v), from about 0.6% (w/v) to about 3% (w/v), from about 0.6% (w/v) to about 4% (w/v), from about 0.6% (w/v) to about 5% (w/v), from about 0.6% (w/v) to about 7% (w/v), from about 0.6% (w/v) to about 9% (w/v), from about 0.6% (w/v) to about 11% (w/v), from about 0.6% (w/v) to about 13% (w/v), from about 0.6% (w/v) to about 15% (w/v), from about 0.7% (w/v) to about 0.8% (w/v), from about 0.7% (w/v) to about 0.9% (w/v), from about 0.7% (w/v) to about 1.0% (w/v), from about 0.7% (w/v) to about 1.5% (w/v), from about 0.7% (w/v) to about 2.0% (w/v), from about 0.7% (w/v) to about 2.5% (w/v), from about 0.7% (w/v) to about 3% (w/v), from about 0.7% (w/v) to about 4% (w/v), from about 0.7% (w/v) to about 5% (w/v), from about 0.7% (w/v) to about 7% (w/v), from about 0.7% (w/v) to about 9% (w/v), from about 0.7% (w/v) to about 11% (w/v), from about 0.7% (w/v) to about 13% (w/v), from about 0.7% (w/v) to about 15% (w/v), from about 0.8% (w/v) to about 0.9% (w/v), from about 0.8% (w/v) to about 1.0% (w/v), from about 0.8% (w/v) to about 1.5% (w/v), from about 0.8% (w/v) to about 2.0% (w/v), from about 0.8% (w/v) to about 2.5% (w/v), from about 0.8% (w/v) to about 3% (w/v), from about 0.8% (w/v) to about 4% (w/v), from about 0.8% (w/v) to about 5% (w/v), from about 0.8% (w/v) to about 7% (w/v), from about 0.8% (w/v) to about 9% (w/v), from about 0.8% (w/v) to about 11% (w/v), from about 0.8% (w/v) to about 13% (w/v), from about 0.8% (w/v) to about 15% (w/v), from about 0.9% (w/v) to about 1.0% (w/v), from about 0.9% (w/v) to about 1.5% (w/v), from about 0.9% (w/v) to about 2.0% (w/v), from about 0.9% (w/v) to about 2.5% (w/v), from about 0.9% (w/v) to about 3% (w/v), from about 0.9% (w/v) to about 4% (w/v), from about 0.9% (w/v) to about 5% (w/v), from about 0.9% (w/v) to about 7% (w/v), from about 0.9% (w/v) to about 9% (w/v), from about 0.9% (w/v) to about 11% (w/v), from about 0.9% (w/v) to about 13% (w/v), from about 0.9% (w/v) to about 15% (w/v), from about 1.0% (w/v) to about 1.5% (w/v), from about 1.0% (w/v) to about 2.0% (w/v), from about 1.0% (w/v) to about 2.5% (w/v), from about 1.0% (w/v) to about 3% (w/v), from about 1.0% (w/v) to about 4% (w/v), from about 1.0% (w/v) to about 5% (w/v), from about 1.0% (w/v) to about 7% (w/v), from about 1.0% (w/v) to about 9% (w/v), from about 1.0% (w/v) to about 11% (w/v), from about 1.0% (w/v) to about 13% (w/v), from about 1.0% (w/v) to about 15% (w/v), from about 1.5% (w/v) to about 2.0% (w/v), from about 1.5% (w/v) to about 2.5% (w/v), from about 1.5% (w/v) to about 3% (w/v), from about 1.5% (w/v) to about 4% (w/v), from about 1.5% (w/v) to about 5% (w/v), from about 1.5% (w/v) to about 7% (w/v), from about 1.5% (w/v) to about 9% (w/v), from about 1.5% (w/v) to about 11% (w/v), from about 1.5% (w/v) to about 13% (w/v), from about 1.5% (w/v) to about 15% (w/v), from about 2.0% (w/v) to about 2.5% (w/v), from about 2.0% (w/v) to about 3% (w/v), from about 2.0% (w/v) to about 4% (w/v), from about 2.0% (w/v) to about 5% (w/v), from about 2.0% (w/v) to about 7% (w/v), from about 2.0% (w/v) to about 9% (w/v), from about 2.0% (w/v) to about 11% (w/v), from about 2.0% (w/v) to about 13% (w/v), from about 2.0% (w/v) to about 15% (w/v), from about 2.5% (w/v) to about 3% (w/v), from about 2.5% (w/v) to about 4% (w/v), from about 2.5% (w/v) to about 5% (w/v), from about 2.5% (w/v) to about 7% (w/v), from about 2.5% (w/v) to about 9% (w/v), from about 2.5% (w/v) to about 11% (w/v), from about 2.5% (w/v) to about 13% (w/v), from about 2.5% (w/v) to about 15% (w/v), from about 3% (w/v) to about 4% (w/v), from about 3% (w/v) to about 5% (w/v), from about 3% (w/v) to about 7% (w/v), from about 3% (w/v) to about 9% (w/v), from about 3% (w/v) to about 11% (w/v), from about 3% (w/v) to about 13% (w/v), from about 3% (w/v) to about 15% (w/v), from about 4% (w/v) to about 5% (w/v), from about 4% (w/v) to about 7% (w/v), from about 4% (w/v) to about 9% (w/v), from about 4% (w/v) to about 11% (w/v), from about 4% (w/v) to about 13% (w/v), from about 4% (w/v) to about 15% (w/v), from about 5% (w/v) to about 7% (w/v), from about 5% (w/v) to about 9% (w/v), from about 5% (w/v) to about 11% (w/v), from about 5% (w/v) to about 15% (w/v), from about 5% (w/v) to about 15% (w/v), from about 7% (w/v) to about 9% (w/v), from about 7% (w/v) to about 11% (w/v), from about 7% (w/v) to about 13% (w/v), from about 7% (w/v) to about 15% (w/v), from about 9% (w/v) to about 11% (w/v), from about 9% (w/v) to about 13% (w/v), from about 9% (w/v) to about 15% (w/v), from about 11% (w/v) to about 13% (w/v), from about 11% (w/v) to about 15% (w/v), from about 13% (w/v) to about 15% (w/v), about 0.1% (w/v), about 0.2% (w/v), about 0.3% (w/v), about 0.4% (w/v), about

0.5% (w/v), about 0.6% (w/v), about 0.7% (w/v), about 0.8% (w/v), about 0.9% (w/v), about 1.0% (w/v), about 1.5% (w/v), about 2.0% (w/v), about 2.5% (w/v), about 3% (w/v), about 4% (w/v), about 5% (w/v), about 7% (w/v), about 9% (w/v), about 11% (w/v), about 13% (w/v), and about 15% (w/v).

The compositions disclosed herein may further comprise a buffering agent. The buffering agent may be an amine or a non-amine compound. In some embodiments, the buffering agent has at least one pK_a selected from the group consisting of: from approximately 7.0 to approximately 9.0, from approximately 7.5 to approximately 9.0, from approximately 8.0 to approximately 9.0, from approximately 8.5 to approximately 9.0, from approximately 7.0 to approximately 8.5, from approximately 7.5 to approximately 8.5, from approximately 8.0 to approximately 8.5, from approximately 7.0 to approximately 8.0, from approximately 7.5 to approximately 8.0, from approximately 7.0 to approximately 7.5, 7.0 or greater, 7.5 or greater, 8.0 or greater, 8.5 or greater, 9.0 or greater, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, and 9.9.

In one embodiment, the amount of buffering agent in the lysis solution is selected from the group consisting of: from about 25 mM to about 50 mM, from about 25 mM to about 75 mM, from about 25 mM to about 100 mM, from about 25 mM to about 125 mM, from about 25 mM to about 130 mM, from about 25 mM to about 135 mM, from about 25 mM to about 140 mM, from about 25 mM to about 145 mM, from about 25 mM to about 150 mM, from about 25 mM to about 155 mM, from about 25 mM to about 160 mM, from about 25 mM to about 165 mM, from about 25 mM to about 170 mM, from about 25 mM to about 175 mM, from about 25 mM to about 200 mM, from about 25 mM to about 225 mM, from about 25 mM to about 250 mM, from about 50 mM to about 75 mM, from about 50 mM to about 100 mM, from about 50 mM to about 125 mM, from about 50 mM to about 130 mM, from about 50 mM to about 135 mM, from about 50 mM to about 140 mM, from about 50 mM to about 145 mM, from about 50 mM to about 150 mM, from about 50 mM to about 155 mM, from about 50 mM to about 160 mM, from about 50 mM to about 165 mM, from about 50 mM to about 170 mM, from about 50 mM to about 175 mM, from about 50 mM to about 200 mM, from about 50 mM to about 225 mM, from about 50 mM to about 250 mM, from about 75 mM to about 100 mM, from about 75 mM to about 125 mM, from about 75 mM to about 130 mM, from about 75 mM to about 135 mM, from about 75 mM to about 140 mM, from about 75 mM to about 145 mM, from about 75 mM to about 150 mM, from about 75 mM to about 155 mM, from about 75 mM to about 160 mM, from about 75 mM to about 165 mM, from about 75 mM to about 170 mM, from about 75 mM to about 175 mM, from about 75 mM to about 200 mM, from about 75 mM to about 225 mM, from about 75 mM to about 250 mM, from about 100 mM to about 125 mM, from about 100 mM to about 130 mM, from about 100 mM to about 135 mM, from about 100 mM to about 140 mM, from about 100 mM to about 145 mM, from about 100 mM to about 150 mM, from about 100 mM to about 155 mM, from about 100 mM to about 160 mM, from about 100 mM to about 165 mM, from about 100 mM to about 170 mM, from about 100 mM to about 175 mM, from about 100 mM to about 200 mM, from about 100 mM to about 225 mM, from about 100 mM to about 250 mM, from about 125 mM to about 130 mM, from about 125 mM to about 135 mM, from about 125 mM to about 140 mM, from about 125 mM to about 145 mM, from about 125 mM to about 150 mM, from about 125 mM to about 155 mM, from about 125 mM to about 160 mM, from about 125 mM to about 165 mM, from about 125 mM to about 170 mM, from about 125 mM to about 175 mM, from about 125 mM to about 200 mM, from about 125 mM to about 225 mM, from about 125 mM to about 250 mM, from about 130 mM to about 135 mM, from about 130 mM to about 140 mM, from about 130 mM to about 145 mM, from about 130 mM to about 150 mM, from about 130 mM to about 155 mM, from about 130 mM to about 160 mM, from about 130 mM to about 165 mM, from about 130 mM to about 170 mM, from about 130 mM to about 175 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from about 165 mM to about 225 mM, from about 165 mM to about 250 mM, from about 170 mM to about 175 mM, from about 170 mM to about 200 mM, from about 170 mM to about 225 mM, from about 170 mM to about 250 mM, from about 175 mM to about 200 mM, from about 175 mM to about 225 mM, from about 175 mM to about 250 mM, from about 200 mM to about 225 mM, from about 200 mM to about 250 mM, from about 225 mM to about 250 mM, about 25 mM, about 50 mM, about 75 mM, about 100 mM, about 125 mM, about 130 mM, about 135 mM, about 140 mM, about 145 mM, about 150 mM, about 155 mM, about 160 mM, about 165 mM, about 170 mM, about 175 mM, about 200 mM, about 225 mM, and about 250 mM.

Exemplary buffering agents include without limitation tris (hydroxymethyl)aminomethane ("TRIS") and derivatives thereof, such as N-tris-(hydroxymethyl)methyl-3-aminopropanesulfonic acid ("TAPS"), 3-[N-tris-(hydroxymethyl)-methyl-amino]-2-hydroxypropanesulfonic acid ("TAPSO"); N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid ("TES"); N-[tris(hydroxymethyl)methyl]-glycine ("TRICINE"); bis(2-hydroxyethyl)iminotris-(hydroxymethyl) methane ("bis-TRIS"); 1,3-bis[tris(hydroxymethyl)methy-

lamino]propane ("bis-TRIS PROPANE"); carbonate-bicarbonate; glycine; phosphate; 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid ("HEPES"); N,N-bis(2-hydroxyethyl)glycine ("Bicine"); 3-(N-morpholino) propanesulfonic acid ("MOPS"); and other Good buffers.

In some embodiments, the lysis solution has a pH selected from the group consisting of: from approximately 7.5 to approximately 10, from approximately 7.5 to approximately 9.5, from approximately 8.0 to approximately 9.5, from approximately 8.5 to approximately 9.5, from approximately 9.0 to approximately 9.5, from approximately 7.5 to approximately 9.0, from approximately 8.0 to approximately 9.0, from approximately 8.5 to approximately 9.0, from approximately 7.0 to approximately 8.5, from approximately 7.5 to approximately 8.5, from approximately 8.0 to approximately 8.5, from approximately 7.0 to approximately 8.0, from approximately 7.5 to approximately 8.0, from approximately 7.0 to approximately 7.5, 7.0 or greater, 7.5 or greater, 8.0 or greater, 8.5 or greater, 9.0 or greater, 9.5 or greater, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, and 10.0.

In another embodiment, the composition further comprises a protein digestive enzyme. By way of example and not limitation, the protein digestive enzyme may be Proteinase K, trypsin, pepsin, thermolysin, thrombin, factor Xa, and combinations thereof.

In another embodiment, the lysis solution comprises a preservative. Suitable preservatives include sodium azide, gentomycin, and ProClin®, which is a composition comprising three isothiazolones: 2-Methyl-4-isothiazolin-3-one, 5-Chloro-2-methyl-4-isothiazolin-3-one, and 1,2-Benzisothiazolin-3-one. In one embodiment, the amount of preservative in the lysis solution can be about 0.01%, or about 0.02%, or about 0.03%, or about 0.04%, or about 0.05%, or about 0.06%, or about 0.07%, or about 0.08%, or about 0.09%, or about 0.10%, or about 0.11%, or about 0.12%, or about 0.13%, or about 0.14%, or about 0.15%, or about 0.16%, or about 0.17%, or about 0.18%, or about 0.19%, or about 0.20%.

In another embodiment, the composition further comprises at least one reagent for isolating nucleic acids. By way of example and not limitation, the reagent for isolating nucleic acids can be magnetic beads modified to bind specifically to nucleic acids.

The present disclosure further provides methods of preparing a fixed biological sample for molecular analysis comprising lysing the fixed biological sample in the presence of composition comprising at least two amines to create a lysate; and isolating a component of the lysate.

Any manner of lysing the fixed biological sample can be used in the disclosed method, including without limitation: mechanical lysis, such as by sonication or cytolysis; and chemical lysis, including use of detergents such as 3-[(3 cholamidopropyl)dimethylammonio]-1-propanesulfonate (sold commercially as CHAPS), NONIDET P-40 (also known as Igepal CA-630), deoxycholate, TRITON X-100 (also known as 4-(1,1,3,3-Tetramethylbutyl)phenyl-polyethylene glycol), sodium dodecyl sulfate (sold commercially as SDS), and/or polysorbate surfactants (sold commercially as TWEEN).

In a further embodiment, the lysis step of the disclosed method is performed in the presence of heat.

In a further embodiment, the composition comprising at least two amines has a basic pH value. In a further embodiment, the pH value is greater than 7.5. In a further embodiment, the pH value is greater than 8. In a further embodiment, the pH value is greater than 8.5. In a further embodiment, the pH value is greater than 9. In a further embodiment, the pH value is between approximately 8.0 and approximately 10. In a further embodiment, the pH value is between approximately 9 and approximately 10. In a further embodiment, the pH value is between approximately 8.5 and approximately 9.5. In a further embodiment, the pH value is between approximately 9 and approximately 9.5.

In a further embodiment, the lysis step is performed in the presence of a protein digestive enzyme. An exemplary protein digestive enzymes includes, but is not limited to, Proteinase K, trypsin, pepsin, thermolysin, thrombin, factor Xa, and combinations thereof.

In one embodiment, the component of the lysate that is isolated is an organelle. Exemplary organelles that may be isolated include, but are not limited to nuclei, ribosomes, plasma membranes, endoplasmic reticulum, mitochondria, Golgi apparatus, lysosomes, vacuoles, and vesicles.

In one embodiment, the component of the lysate that is isolated is a nucleic acid. Any form of nucleic acid can be recovered using the disclosed methods and reagents, including but not limited to nuclear DNA, mitochondrial DNA, mRNA, chromatin, chromosomal DNA, exogenous plasmids, viral DNA, viral RNA, bacterial DNA, and bacterial RNA.

Nucleic acid recovery methods include without limitation: chromatography, including but not limited to silica or glass adsorption, ion exchange chromatography, affinity purification, spin column chromatography, and gel filtration; solvent extraction and precipitation; and centrifugation. Nucleic acid recovery methods include without limitation ammonium sulfate precipitation, differential solubilization, sucrose gradient centrifugation, and chromatography. By way of example and not limitation, the nucleic acid may be isolated by using magnetic beads modified to bind specifically to nucleic acids.

In another embodiment, a nucleic acid comprising a specific sequence may be isolated by hybridizing it to a nucleic acid probe complementary to the specific sequence. In one embodiment, the nucleic acid probe is bound to a solid phase or adapted to be bound to a solid phase. In another embodiment, hybridization of the nucleic acid probe to the nucleic acid molecule results in a DNA:RNA hybrid between the probe and the nucleic acid molecule. The resulting hybrid may then be bound by an antibodies known to bind specifically to DNA:RNA hybrids ("DNA:RNA-binding antibody"), which in turn may be bound to a solid phase or

adapted to be bound to a solid phase. In either case, hybridization of the probe with the nucleic acid results in the nucleic acid being associated with a solid phase, which may then be separated from the lysate using mechanical means. By way of example and not limitation, such methods are described in U.S. Pat. No. 6,228,578 and U.S. patent application Ser. No. 12/695,071, the contents of which are incorporated in their entirety by reference. Exemplary DNA:RNA-binding antibodies include, but are not limited to, those disclosed in U.S. Pat. Nos. 4,732,847 and 4,865,980, the contents of which are incorporated herein by reference in their entireties.

By way of example, and not limitation, an appropriate solid phase includes, but is not limited to: silica, borosilicates, silicates, anorganic glasses, organic polymers such as poly (meth)acrylates, polyurethanes, polystyrene, agarose, polysaccharides such as cellulose, metal oxides such as aluminum oxide, magnesium oxide, titanium oxide and zirconium oxide, metals such as gold or platinum, agarose, sephadex, sepharose, polyacrylamide, divinylbenzene polymers, styrene divinylbenzene polymers, dextrans, and derivatives thereof, and/or silica gels, beads, membranes, and resins; glass or silica surfaces, such as beads, plates, and capillary tubes; magnetizable or magnetic (e.g. paramagnetic, superparamagnetic, ferromagnetic or ferrimagnetic) particles, including but not limited to polystyrene, agarose, polyacrylamide, dextran, and/or silica materials having a magnetic material incorporated therein or associated therewith. In some exemplary embodiments, the nucleic acid probe or antibody can be linked to the surface of a processing vessel such as a micro-tube, a well of micro-plate, or capillary, and using these surfaces the nucleic acid can be isolated on a micro scale. Where a biotinylated nucleic acid probe or antibody is provided, the solid phase may be coated with a substance capable of binding the biotin moiety, such as, for example, avidin, streptavidin, and/or neutravidin. In another embodiment, the solid phase may be coated with, or adapted to be coated with, an antibody specific for a DNA:RNA hybrid.

Nucleic acids obtained using the disclosed methods and compositions may be used in subsequent molecular analytical methods including without limitation gel electrophoresis, PCR-related techniques including reverse transcriptase PCR and real time PCR, sequencing, sub-cloning procedures, Southern blotting, northern blotting, fluorescent in situ hybridization, and various mutational analyses including hybrid capture and multiplex analysis.

In one embodiment, the component of the lysate that is isolated is a protein. Protein recovery methods include without limitation ammonium sulfate precipitation, differential solubilization, sucrose gradient centrifugation, and chromatography. Chromatographic protein isolation methods include without limitation size exclusion, ion exchange, hydrophobic interaction, affinity, immuno-affinity, and metal binding chromatography.

Proteins obtained with the disclosed methods and compositions may be used in subsequent molecular analytical methods including without limitation sequencing, immunoprecipitation, western blots, ELISA assays, dot blots, and enzyme assay

The methods described also can be used to isolate pathogens, including without limitation bacteria, fungi, yeast, protozoa, prions, and viruses.

The methods and compositions described herein are easily and rapidly optimized for specimens preserved in either cross-linking or precipitating fixatives.

The methods and compositions described herein also are adaptable for all biological fluids and provide to simple protocols that are proven compatible with high throughput automation, including for example the QlAensemble® Next Gen™ Sample Processor, an automated sample processing device for extraction and analysis which provides full automation, including de-capping and capping of specimens and zero ergonomic movements. As such, they provide for ultra high through-put and ecologically friendly sample processing by allowing for a flexible input volume, non-hazardous material liquid waste, limited solid waste, and reagents that may be stored at room temperature.

EXAMPLES

Example 1

This following example shows the effect of various lysis solutions on the yield and signal sensitivity of HPV DNA isolated from aldehyde-fixed clinical cervical samples.

Clinical cervical samples were collected and fixed in SUREPATH fixative. The fixed samples were then washed and suspended in a lysis solution ("LB") of: (1) 3% (v/v) Brij-58, and (2) 150 mM Tris-HCl. An additional amine selected from the following group was added to test samples at a concentration of 200 mM: diethanolamine ("DEA"), triethanolamine ("TEA"), TEA-HCl, triethylamine ("TE"), DEA plus indium (III) chloride ("IC"), dicyandiamide ("DC"), DEA plus magnesium perchlorate ("$Mg(ClO_4)_2$"); hexamethylene-tetramine ("HMTA"), DEA plus palladium (II) acetate ("PA"), diethylenetriamine ("DETA"), ethylenediamine ("EDA"), glycine, hydroxylamine ("HA"), and ammonium chloride. Typically, 1.5 mL of the sample is added to 1 mL of lysis buffer, plus 25 µl of Proteinase K (10 mg/ml stock) and 60 µl of 1.5% (v/v) AxpH™ DNA-affinity magnetic beads to lyse. A magnetic field was applied to the tubes and the lysate was removed, leaving only the magnetic beads. DNA was eluted from the beads and the presence of HPV DNA was determined using a hybrid capture method as described in U.S. Pat. No. 6,228,578, the contents of which are incorporated in their entirety by reference. Results are shown in Tables 1 and 2 and FIGS. **1**A and **1**B. Shaded cells in tables 1 and 2 indicate replicates wherein RLU/CO is greater than 1.00.

Tables 1 and 2 show raw data from each replicate (RLU/CO) and combined data for each lysis solution tested. The combined data set is displayed graphically at FIGS. **1**A and **1**B. As can be seen, the addition of an amine-containing compound to a Tris-buffered lysis solution increased the sensitivity of detection of the HPV DNA in the cervical samples.

TABLE 1

	LB	LB + DEA	LB + TEA	LB + TEA - HCl	LB + TE	LB + DEA - IC	LB + DC	LB + DEA + $Mg(ClO_4)_2$	LB + HMTA	LB + DEA + PA
pH	6.890	8.700	8.060	6.400	10.050	7.530	6.910	8.750	6.990	8.470
RLU/CO	0.20	1.46	7.19	0.81	1.25	0.29	2.92	2.52	1.08	0.15
	0.70	2.16	14.29	1.00	6.61	0.28	1.52	3.14	0.57	0.18
	0.55	2.69	1.86	0.54	0.50	0.25	0.89	0.60	0.34	0.19
	2.15	9.09	1.40	0.67	0.55	0.24	1.22	0.74	1.06	0.21
	0.74	6.98	2.41	1.28	0.57	0.18	1.52	0.54	1.31	0.17
	0.44	11.58	6.08	0.55	0.48	0.23	3.70	0.47	2.92	0.21
	0.95	4.46	2.69	1.13	1.27	0.18	0.64	2.88	0.80	0.16
	0.50	11.59	14.22	1.82	0.41	0.15	5.22	2.01	0.56	0.20
Ave. RLU	169	1354	1357	211	315	49	477	349	234	40
Std. Dev. RLU	129	900	1155	94	457	11	346	248	175	5
CV %	76.5%	66.5%	85.1%	44.7%	145.1%	21.6%	72.7%	71.0%	74.8%	13.8%
Ave. RLU/CO	0.78	6.25	6.27	0.97	1.45	0.23	2.20	1.61	1.08	0.18
Std. Dev. of RLU/CO	0.60	4.16	5.34	0.44	2.11	0.05	1.60	1.15	0.81	0.03
Fold increase	1.00	8.01	8.03	1.25	1.86	0.29	2.82	2.07	1.39	0.24

TABLE 2

	LB	LB + EA	LB + EA HCl	LB + EDA	LB + DETA	LB + glycine	LB + DEA	LB + DEA HCl	LB + HA	LB + Ammonium chloride
pH	6.930	9.310	7.000	9.680	9.860	6.970	8.680	7.040	7.110	6.950
RLU/CO	0.36	7.73	0.33	0.53	2.45	0.89	1.50	0.33	1.19	1.29
	0.91	1.07	1.43	17.95	2.03	3.20	0.68	0.33	9.62	0.50
	0.46	8.96	0.72	2.28	1.66	0.96	1.29	0.87	1.10	0.83
	2.77	1.49	0.26	10.58	0.51	0.56	2.89	2.52	1.37	0.26
	0.28	2.48	0.40	10.50	1.30	0.51	0.46	0.62	1.12	1.17
	0.67	5.59	1.03	3.24	3.01	0.87	4.53	0.71	0.89	0.54
	0.43	6.88	0.79	7.27	6.91	0.40	1.23	1.08	1.24	1.28
	0.74	1.37	0.39	1.03	0.58	0.31	2.47	1.85	0.44	2.43
Ave. RLU	216	1158	174	1738	601	251	490	271	553	270
Std. Dev. of RLU	212	834	106	1581	534	243	351	201	792	178
CV %	98.2%	72.0%	61.1%	91.0%	88.8%	97.0%	71.7%	74.4%	143.3%	65.9
Ave. RLU/CO	0.83	4.45	0.67	6.67	2.31	0.96	1.88	1.04	2.12	1.04
Std. Dev. of RLU/CO	0.81	3.20	0.41	6.07	2.05	0.93	1.35	0.77	3.04	0.68

In addition, Table 3 shows the effect of addition of 200 mM diethanolamine on 26 individual clinical cervical samples. In each case, the addition of diethanolamine led to an increase in the sensitivity, ranging from a 1.01-fold increase to a 13.46-fold increase.

TABLE 3

SP Sample ID	no DEA	200 mM DEA	Fold Increase
SP 005657	46.41	406.20	8.75
SP 005514	1.17	1.44	1.23
SP 005864	0.48	4.55	9.49
SP 005609	126.36	203.74	1.61
SP 005618	190.33	316.37	1.66
SP 005520	361.16	366.03	1.01
SP 005548	12.94	32.98	2.55
SP 005513	207.63	238.05	1.15
SP 005572	117.53	203.80	1.73
SP 005554	8.37	11.49	1.37
SP 005567	1.53	20.52	13.46
SP 005828	5.66	13.84	2.45
SP 005614	5.40	17.14	3.17

TABLE 3-continued

SP Sample ID	no DEA	200 mM DEA	Fold Increase
SP 005586	332.09	495.56	1.49
SP 005594	6.00	34.68	5.78
SP 005495	15.33	25.80	1.68
SP 005569	139.73	261.65	1.87
SP 005755	0.55	2.79	5.08
SP 005470	363.91	604.78	1.66
SP 005475	322.91	505.12	1.56
SP 005477	165.55	243.46	1.47
SP 005399	42.07	116.67	2.77
SP 005611	0.85	6.86	8.11
SP 005482	2.34	3.90	1.67
SP 006011	1.44	7.81	5.43
SP 006014	1.02	1.52	1.49

Example 2

This example shows the effect that varying the concentration of Tris has on the increased efficiency of lysis solution comprising both Tris and diethanolamine.

Analysis was performed in substantially the same was as in Example 1, except the lysis solution comprised (1) 3% (v/v) Brij-58; (2) 300 mM diethanolamine; and (3) Tris at a concentration selected from 50 mM, 100 mM, 150 mM, 200 mM, 250 mM, 300 mM, 350 mM, 400 mM, and 450 mM. Typically, 1.5 mL of the sample is added to 1 mL of lysis buffer, plus 25 μl of Proteinase K (10 mg/ml stock) and 60 μl of 1.5% (v/v) AxpH™ DNA-affinity magnetic beads to lyse. The lysis solution comprising 50 mM TRIS was selected as a baseline for analysis. Results are shown in FIG. **2** and below at Table 4. Shaded cells in Table 4 indicate replicates having an RLU/CO of greater than or equal to 110.

TABLE 4

Tris HCL (mM)	50	100	150	200	250	300	350	400	450	60° C. 60′ lysis CtL
RLU/CO	94.66	109.10	138.98	111.88	63.49	80.12	115.31	92.74	79.73	128.37
	107.81	107.47	121.70	107.62	79.80	89.76	84.62	76.55	90.95	148.43
	105.69	126.75	137.94	122.48	94.77	134.29	77.53	102.08	101.26	134.94
	120.55	169.81	159.89	130.26	84.07	105.06	118.02	114.87	88.02	166.88
	112.54	143.78	120.35	142.79	113.92	84.82	82.62	71.25	78.39	176.96
	138.73	166.23	151.19	118.31	109.72	101.10	108.73	90.80	86.80	214.95
	99.74	145.48	149.31	104.13	105.09	99.09	137.76	106.05	90.94	129.11
	102.73	140.32	137.34	112.38	135.85	106.70	115.33	83.45	95.00	139.74
Ave. RLU	35574	44705	45017	38291	31714	32288	33859	29743	28665	49952
Std. Dev. of RLU	4494	7508	4461	4134	7314	5422	6831	4824	2432	9647
CV %	12.6%	16.8%	9.9%	10.8%	23.1%	16.8%	20.2%	16.2%	8.5%	19.3%
Ave. RLU/CO	110.31	138.62	139.59	118.73	98.34	100.12	104.99	92.22	88.88	154.89
Std. Dev. of RLU	13.94	23.28	13.83	12.82	22.68	16.81	21.18	14.96	7.54	29.91
Fold increase	1.00	1.26	1.27	1.08	0.89	0.91	0.95	0.84	0.81	1.40

Example 3

This example shows the effect of varying the pH on the sensitivity efficiency of nucleic acid lysis solutions comprising both TRIS and diethanolamine.

Analysis was performed in substantially the same way as in Example 1, except the lysis solution comprised (1) 3% (v/v) Brij-58; (2) 300 mM diethanolamine; and (3) 150 mM Tris. Typically, 1.5 mL of the sample is added to 1 mL of lysis buffer, plus 250 of Proteinase K (10 mg/ml stock) and 600 of 1.5% (v/v) AxpH™ DNA-affinity magnetic beads to lyse. The pH of the lysis solution was adjusted to a value of 7.077, 7.397, 8.113, 8.492, 9.021, 9.265, and 9.443. Results are shown in FIG. **3** and Table 5. Shaded cells in Table 5 indicate individual replicates with an RLU/CO of greater than or equal to 2.00. As can be seen, increasing the pH value of the lysis solution increased the sensitivity of HPV DNA detection.

TABLE 5

pH	7.007	7.397	8.113	8.492	9.021	9.265	9.443
RLU/	1.31	3.49	1.49	1.27	4.25	7.07	11.72
CO	1.31	2.89	1.73	1.63	3.24	10.17	4.59
	2.73	2.01	2.53	7.67	2.01	7.64	5.48
	1.89	2.93	2.50	5.20	3.06	11.25	8.47
	0.86	1.08	2.17	3.51	8.45	5.10	5.23
	3.95	4.47	8.75	3.14	4.54	2.78	15.16
	2.70	1.16	1.52	3.42	1.93	13.18	11.50
	1.09	1.00	9.08	8.86	5.10	5.75	12.90
Ave. RLU	716	861	1346	1568	1472	2844	3391
Std. Dev. of RLU	384.8	460.7	1168.9	985.3	761.2	1250.4	1446.0
CV %	53.8%	53.5%	86.9%	62.8%	51.7%	44.0%	42.6%
Ave. RLU/CO	1.98	2.38	3.72	4.34	4.07	7.87	9.38
Std. Dev. of RLU/CO	1.06	1.27	3.23	2.73	2.11	3.46	4.00

Example 4

This example shows the effect of varying the concentration of amine on the sensitivity and efficiency of nucleic acid isolation using a lysis solution comprising both Tris and diethanolamine.

Analysis was performed in substantially the same way as in Example 1, except the lysis solution comprised (1) 3% (v/v) Brij-58; (2) 150 mM Tris; and (3) diethanolamine at a concentration selected from: 0 mM, 39 mM, 78 mM, 156 mM, 312.5 mM, 625 mM, 1250 mM, and 2500 mM. The control (CTL) comprised only buffer. Typically, 1.5 mL of the sample is added to 1 mL of lysis buffer, plus 25 μl of Proteinase K (10 mg/ml stock) and 60 μl of 1.5% (v/v) AxpH™ DNA-affinity magnetic beads to lyse. Results are shown in FIG. **4** and Table 6.

TABLE 6

DEA Conc.	mM	0	39	78	156	312.5	625	1250	2500	60° C. 60′ lysis CtL
	%	0	0.45	0.90	1.80	3.60	7.20	14.40	28.80	
pH		7.983	8.391	8.765	9.164	9.560	9.868	10.191	10.502	6.990
RLU/CO		2.28	8.59	26.65	7.40	14.38	8.14	6.82	2.64	34.41
		4.84	9.50	7.84	13.24	9.60	7.03	2.63	0.60	13.40
		6.09	6.22	8.65	12.89	6.95	3.47	3.25	2.38	35.47
		11.03	3.15	7.11	12.49	12.89	1.36	6.69	0.90	36.74
		3.19	9.66	30.22	17.19	7.60	7.77	1.69	1.95	25.95
		3.73	12.29	12.20	5.28	16.63	4.52	2.17	1.76	47.88
		5.41	6.58	16.11		15.75	9.06	9.94	0.77	12.52
		4.73	15.22	7.77	12.37	12.06	4.15	2.76	1.65	26.90
Ave. RLU		1360	2346	3840	3044	3158	1499	1184	417	7684
Std. Dev. of RLU		703	986	2401	1046	958	711	779	200	3174
CV %		51.7%	42.0%	62.5%	34.4%	30.3%	47.4%	65.8%	47.9%	41.3%
Ave. RLU/CO		5.16	8.90	14.57	11.55	11.98	5.69	4.49	1.58	29.16
Std. Dev. of RLU/CO		2.67	3.74	9.11	3.97	3.64	2.70	2.96	0.76	12.05

Example 5

This example shows the relative contributions of detergent and each amine. Analysis was performed in substantially the same way as in Example 1, except seven lysis solutions were used: (1) 3% (v/v) Brij-58; (2) 150 mM Tris-HCl; (3) 150 mM diethanolamine; (4) Brij-58 plus 150 mM Tris-HCl; (5) Brij-58 plus 150 mM diethanolamine; (6) 150 mM Tris plus 150 mM diethanolamine; and (7) Brij-58 plus 150 mM Tris plus 150 mM diethanolamine. The control (CTL) comprised only buffer. Typically, 1.5 mL of the sample is added to 1 mL of lysis buffer, plus 25 μl of Proteinase K (10 mg/ml stock) and 60 μl of 1.5% (v/v) AxpH™ DNA-affinity magnetic beads to lyse. Results are shown in FIG. **5** and at Table 7. Shaded cells in Table 7 indicate replicates having an RLU/CO of greater than 1.00.

TABLE 7

		Brij58	Tris-HCl, 7.5	DEA	Tris + Brij58	DEA + Brij58	Tris + DEA	Brij58 Tris + DEA	60° C. 60′ lysis CtL
pH	LB + SP + Beads	7.15	6.75	10.11	6.93	10.11	9	9.01	9.2
	PAS LB	8.09	7.53	11.21	7.51	11.18	9.22	9.19	9.4
RLU/CO		1.10	6.49	0.72	0.97	0.71	7.31	2.94	5.19
		0.55	6.07	0.41	1.50	0.34	3.44	1.99	16.86
		0.70	3.01	0.60	1.30	0.59	3.74	1.33	4.96
		1.02	1.11	0.48	1.67	0.37	4.47	8.35	2.74
		2.82	1.12	0.48	2.05	0.34	3.91	9.08	
		2.52	2.79	0.42	1.27	0.60	4.44	32.88	
		0.97	10.22	0.59	4.98	0.35	2.56	7.49	
		0.89	1.24	0.42	2.08	0.23	1.97	7.24	
Ave. RLU		270	818	105	403	90	811	1818	1517
Std. Dev. of RLU		174	671	23	259	35	327	2070	1160
CV %		64.6%	82.1%	22.1%	64.3%	38.5%	40.3%	113.9%	76.5%
Ave. RLU/CO		1.32	4.01	0.51	1.98	0.44	3.98	8.91	7.44
Std. Dev. of RLU/CO		0.85	3.29	0.11	1.27	0.17	1.60	10.15	6.38
Fold increase		1.00	3.03	0.39	1.49	0.33	3.01	6.74	

Example 6

This example shows that the compositions and methods disclosed herein can be used with biological samples preserved using either cross-linking fixatives or precipitating fixatives.

Two types of liquid cryological preservative media are commonly used to preserve clinical cervical samples: SUREPATH, which is an aldehyde-based fixative; and PRESERVCYT, which is a methanol-based fixative. Methods have been developed for testing such samples for HPV DNA, the presence of which is indicative of an active HPV infection. Previously, no uniform method had been developed that is useful for both SUREPATH and PRESERVCYT-fixed clinical cervical samples.

The presently-disclosed methods and compositions were tested for their utility in detecting HPV DNA in samples fixed in either SUREPATH or PRESERVCYT.

SiHa cells are a squamous cell carcinoma cell line derived from a patient having grade II cervical tumor. SiHa cells have been shown to contain an integrated HPV-type 16 genome and thus provide a useful positive control for the extraction and detection of HPV DNA.

SiHa cells were spiked into an HPV-negative clinical specimen pool and preserved in either SUREPATH or PRESERVCYT. The same volume of HPV-negative clinical specimen pool lacking SiHa cells were used as controls. Each sample was pelleted by centrifugation and the supernatant decanted.

One set of each sample was then extracted using a commercially available method by suspending the cell pellet in 50 μL Specimen Transport Medium comprising guanidine hydrochloride and 25 μL of Denaturation Regent comprising NaOH and then lysed at 65° C. for 90 min.

A second set of each sample was resuspended in deionized water. 3% (v/v/) Brij-58, 150 mM Tris, 150 mM diethanolamine, DNA binding magnetic beads, and Proteinase K were added to the suspension and the sample was then lysed at 68.5° C. for 7.5 minutes and then 60° C. for 12.5 minutes. A magnetic field was applied to separate the beads from the solution, then the beads were washed, and DNA eluted.

DNA eluates generated by both methods then were then tested side-by-side by a hybrid capture method. Recovery was determined by signal output from each method. A flow chart outlining the two methods can be seen at FIG. **6**. Results can be seen at FIG. **7**. In PRESERVCYT samples, both recovery methods displayed a similar degree of DNA recovery. In SUREPATH samples, on the other hand, use of a Brij-58/Tris/diethanolamine lysis solution resulted in a 3.5 fold increase in signal compared with the standard lysis solution.

Various detergents also were tested in these methods. SiHa cells were spiked into an HPV-negative clinical specimen pool and preserved in either SUREPATH or PRESERVCYT. The same volume of HPV-negative clinical specimen pool lacking SiHa cells were used as controls. Each sample was pelleted by centrifugation at and the supernatant decanted. The cell pellet was then resuspended in 1.5 mL of deionized water. A lysis solution comprising 150 mM Tris, 150 mM diethanolamine, and a detergent chosen from 3% (v/v) Brij-58, Tween-20, and TRITON X-100) was used. Typically, 1.5 mL of the sample is added to 1 mL of lysis buffer, plus 25 μl of Proteinase K (10 mg/ml stock) and 60 μl of 1.5% (v/v) AxpH™ DNA-affinity magnetic beads to lyse. The sample was then lysed at 68.5° C. for 7.5 minutes and then 60° C. for 12.5 min. A magnetic field was applied to separate the beads from the solution, the beads were washed, and DNA eluted.

Results are shown at FIGS. **8**A to **8**D. As can be seen, there is no significant difference in the amount DNA recovered based on the identity of the detergent.

What is claimed is:

1. A method of lysing a fixed biological sample, the method comprising lysing the fixed biological sample with a composition comprising at least two amines, wherein the method is useful for both aldehyde-based fixatives and alcohol-based fixatives, and wherein the pH of the composition is 8 or greater.

2. A method of isolating a component of a fixed biological sample, the method comprising:

a. lysing the fixed biological sample according to the method of claim **1**; and

b. isolating the component from the lysate.

3. The method of claim **2**, wherein at least one of the amines is selected from the group consisting of methylamine, dimethylamine, diethylamine, trimethlylamine, triethylamine, monoethanolamine, diethanolamine, triethanolamine, ethylenediamine, diethylenetriamine, tris(hydroxymethyl)aminomethane, hexamethylenetetramine, aniline, and an amino acid.

4. The method of claim **2**, wherein at least one of the amines is a buffering agent.

5. The method of claim **4**, wherein the buffering agent is selected from the group consisting of tris(hydroxymethyl) aminomethane ("TRIS"), N-tris-(hydroxymethyl)methyl-3-aminopropanesulfonic acid ("TAPS"), 3-[N-tris-(hydroxymethyl)-methyl-amino]-2-hydroxypropanesulfonic acid ("TAPSO"); N-tris(hydroxymethyl)methyl-2-am inoethanesulfonic acid ("TES"); N-[tris(hydroxymethyl)methyl]-glycine ("TRICINE"); bis(2-hydroxyethyl)iminotris-(hydroxymethyl)methane ("bis-TRIS"); and 1,3-bis[tris (hydroxy-methyl)methylamino]propane ("bis-TRIS PROPANE").

6. The method of claim **4**, wherein the buffering agent is TRIS.

7. The method of claim **2**, wherein at least one of the amines is diazoamine.

8. The method of claim **2**, wherein the composition further comprises at least one detergent.

9. The method of claim **8**, wherein the detergent is selected from the group consisting of polyoxyethyleneglycol dodecyl ether, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, tert-octylphenoxy poly(oxyethylene)ethanol (NONIDET P-40), Igepal CA-630, deoxycholate, 4-(1,1,3,3-Tetramethylbutyl)phenyl-polyethylene glycol (TRITON X-100), sodium dodecyl sulfate, and polysorbate surfactants.

10. The method of claim **2**, wherein the composition further comprises at least one DNA-binding magnetic bead.

11. The method of claim **2**, wherein the composition comprises:

150 mM Tris-HCl;

300 mM diethanolamine;

3% Brij-58;

0.09% sodium azide; and

wherein the composition has a pH of approximately 9.4.

12. The method of claim **1**, wherein the fixed biological sample is fixed with a cross-linking fixative agent.

13. The method of claim **12**, wherein the cross-linking fixative agent comprises an aldehyde.

14. The method of claim **13**, wherein the aldehyde is selected from the group consisting of formaldehyde and glutaraldehyde.

15. The method of claim **1**, wherein the fixed biological sample is fixed with a fixative agent comprising an alcohol.

16. The method of claim 15, wherein the alcohol is selected from the group consisting of methanol and ethanol.

17. The method of claim 1, wherein the fixed biological sample is fixed with a fixative agent selected from the group consisting of the aldehyde-based cytology medium SUREPATH™, and the alcohol-based cytology medium PRESERVCYT™.

18. The method of claim 1, wherein the fixed biological sample is a cervical sample.

19. The method of claim 2, wherein the component is a nucleic acid molecule.

20. The method of claim 19, wherein the nucleic acid molecule comprises a specific sequence.

21. The method of claim 20, wherein the nucleic acid is isolated according to a method comprising hybridizing a nucleic acid probe to the specific sequence of the nucleic acid molecule.

22. The method of claim 21, wherein the nucleic acid probe is adapted to be bound to a solid phase or is bound to a solid phase.

23. The method of claim 22, wherein the solid phase is a magnetic bead.

24. The method of claim 21, wherein the hybridizing of the nucleic acid probe to the specific sequence of the nucleic acid molecule results in the formation of a DNA:RNA hybrid between the nucleic acid molecule and the nucleic acid probe.

25. The method of claim 24, wherein the DNA:RNA hybrid is bound by a DNA:RNA hybrid-binding antibody.

26. The method of claim 25, wherein the DNA:RNA hybrid-binding antibody is bound to a solid phase or adapted to be bound to a solid phase.

27. The method of claim 26, wherein the solid phase is a magnetic bead.

28. The method of claim 19, wherein the nucleic acid molecule is a viral nucleic acid molecule.

29. The method of claim 2, wherein the fixed biological sample is a cervical sample.

30. The method of claim 9, wherein the detergent is deoxycholate, 4-(1,1,3,3-Tetramethylbutyl)phenyl-polyethylene glycol (TRITON X-100).

31. The method of claim 2, wherein the composition comprises Tris-HCl, diethanolamine, Brij-58, and sodium azide.

32. The method of claim 31, wherein the composition has a pH of approximately 9.4.

33. The method of claim 31, wherein the amount of each of Tris-HCl and diethanolamine is about 150 mM or greater.

* * * * *